Neuroscience 340 (2017) 373–383

STREPTOZOTOCIN INDUCED OXIDATIVE STRESS, INNATE IMMUNE SYSTEM RESPONSES AND BEHAVIORAL ABNORMALITIES IN MALE MICE

SHAYAN AMIRI, [a,b,c†] **ARYA HAJ-MIRZAIAN,** [a,b†] **MAJID MOMENY,** [d] **HOSSEIN AMINI-KHOEI,** [b,e] **MARYAM RAHIMI-BALAEI,** [f] **SIMIN POURSAMAN,** [a] **MOJGAN RASTEGAR,** [c] **VAHID NIKOUI,** [g] **TAHMINEH MOKHTARI,** [h] **MAHMOUD GHAZI-KHANSARI** [a,b*] **AND MIR-JAMAL HOSSEINI** [i,j*]

[a] *Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

[b] *Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran*

[c] *Regenerative Medicine Program, Department of Biochemistry and Medical Genetics, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada*

[d] *Hematology/Oncology and Stem Cell Transplantation Research Center, Shariati Hospital, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

[e] *Department of Physiology and Pharmacology, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran*

[f] *Department of Human Anatomy and Cell Science, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada*

[g] *Razi Drug Research Center, Iran University of Medical Sciences, Tehran, Iran*

[h] *Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

[i] *Zanjan Applied Pharmacology Research Center, Zanjan University of Medical Sciences, Zanjan, Iran*

[j] *Department of Pharmacology and Toxicology, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran*

Abstract—Recent evidence indicates the involvement of inflammatory factors and mitochondrial dysfunction in the etiology of psychiatric disorders such as anxiety and depression. To investigate the possible role of mitochondrial-induced sterile inflammation in the co-occurrence of anxiety and depression, in this study, we treated adult male mice with the intracerebroventricular (i.c.v.) infusion of a single low dose of streptozotocin (STZ, 0.2 mg/mouse). Using valid and qualified behavioral tests for the assessment of depressive and anxiety-like behaviors, we showed that STZ-treated mice exhibited behaviors relevant to anxiety and depression 24 h following STZ treatment. We observed that the co-occurrence of anxiety and depressive-like behaviors in animals were associated with abnormal mitochondrial function, nitric oxide overproduction and, the increased activity of cytosolic phospholipase A_2 ($cPLA_2$) in the hippocampus. Further, STZ-treated mice had a significant upregulation of genes associated with the innate immune system such as toll-like receptors 2 and 4. Pathological evaluations showed no sign of neurodegeneration in the hippocampus of STZ-treated mice. Results of this study revealed that behavioral abnormalities provoked by STZ, as a cytotoxic agent that targets mitochondria and energy metabolism, are associated with abnormal mitochondrial activity and, consequently the initiation of innate-inflammatory responses in the hippocampus. Our findings highlight the role of mitochondria and innate immunity in the formation of sterile inflammation and behaviors relevant to anxiety and depression. Also, we have shown that STZ injection (i.c.v.) might be an animal model for depression and anxiety disorders based on sterile inflammation.

Key words: streptozotocin (STZ), depression, anxiety, mitochondria, sterile inflammation, innate immunity.

INTRODUCTION

Major depression is a debilitating disorder that is considered as a health concern in the current century (Ustun, 2001). Despite the enormous efforts that have been done to surmount the obstacles, less progress has been achieved due to treatment or finding the underlying mechanisms of depression (Fournier et al., 2010). Recent evidence indicates the involvement of the immune-inflammatory responses in the pathobiology of depression (Maes, 2011). A large body of evidence has shown that the administration of lipopolysaccharide (LPS) is able to activate toll-like receptor 4 (TLR-4) which, consequently provokes behavioral abnormalities similar to those observed in depressed people (Yirmiya, 1996; Reichenberg et al.,

*Correspondence to: Mahmoud Ghazi-Khansari, Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran. Fax: +98-2166402569. Mir-Jamal Hosseini, Zanjan Applied Pharmacology Research Center, Zanjan University of Medical Sciences, Zanjan, Iran. Fax: +98-2433473639.
E-mail addresses: ghazikha@sina.tums.ac.ir, khansagm@gmail.com (M. Ghazi-Khansari), jamal_hossini@yahoo.com (M.-J. Hosseini).
† Authors contributed equally to this work.

Abbreviations: AD, Alzheimer's disease; ANOVA, analysis of variance; DAMPs, damage-associated molecular patterns; DCFH-DA, 2′,7′-dichlorofluorescein diacetate; FST, Forced swimming test; GSH, Glutathione; H&E, hematoxylin and eosin; HBT, Hole-board test; HPA, hypothalamic–pituitary–adrenal; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; OFT, Open-field test; PLA2, phospholipase A2; ROS, reactive oxygen species; STZ, streptozotocin; TLR-4, toll-like receptor 4.

http://dx.doi.org/10.1016/j.neuroscience.2016.11.003
0306-4522/

2001). Emerging lines of research suggest that NOD-like receptors, the nucleotide-binding oligomerization domain (NOD)-like receptors and TLRs are tightly involved in the pathogenesis of the majority of mental disorders such as depression (Choi and Ryter, 2014).

Regardless of the ability of invasive pathogens to stimulate the innate immune system, activation of immune-inflammatory pathways occurs in response to the cellular stress or damage under sterile conditions. Sterile inflammation is induced as a consequence of trauma, cellular injury or stress which, occurs in the absence of any microorganisms. Immuno-inflammatory responses to sterile inflammation are accompanied by the activation of immune cells and the production of pro-inflammatory cytokines and chemokines (Chen and Nuñez, 2010a,b). Incidence of sterile inflammation is highly relevant to impaired energy hemostasis and mitochondrial dysfunction. Recent evidence suggests that a substantial increase in the production of reactive oxygen species (ROS) (in) directly is implicated in the activation of immune-inflammatory responses through the generation of damage-associated molecular patterns (DAMPs) (Gurung et al., 2015). In this context, a growing body of evidence indicates that mitochondrial dysfunction and inflammatory pathways contribute to the pathobiology of type 2 Diabetes (T2D), Alzheimer's disease (AD) and depression.

Streptozotocin (STZ) is a well-characterized compound to induce oxidative stress, neuro-inflammation and cellular energy dysfunction (Rai et al., 2014; Rajasekar et al., 2014). Although STZ has been widely used for the modeling of diabetes and AD in rodents, a recent study has revealed that the intracerebroventricular (i.c.v.) infusion of STZ caused depressive-like behaviors in mice (Souza et al., 2013b). Considering the involvement of mitochondrial dysfunction and inflammation in the pathogenesis of diabetes, AD and depression, we assumed that the central administration of STZ may be a suitable tool to investigate the underlying mechanisms involved in the pathophysiology of depression under sterile conditions. In this regard, we tested whether (1) i.c.v. administration of STZ is able to provoke behaviors related to the anxiety and depression (2) behavioral changes are associated with mitochondrial dysfunction in the hippocampus and (3) i.c.v. administration of STZ is able to alter immune-inflammatory factors in the hippocampus. We investigated the effects of STZ on the hippocampus because hippocampal formation is highly involved in the pathophysiology of several neurological and psychiatric disorders.

EXPERIMENTAL PROCEDURES

Animals and treatments

Male NMRI mice weighing 25–30 g were purchased from the Pasteur Institute, Tehran, Iran. Animals were housed at the temperature of 21–23 °C under a 12 h regular light/dark cycle with given access to food and water ad lib. All experiments were performed between 10:00 and 14:00 h. All procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals which were approved by the Animal Ethics committee of Zanjan University of Medical Sciences. Full efforts were made to minimize the use of animals and to optimize their comfort.

Streptozotocin treatment

Streptozotocin (Sigma, St Louis, MO, USA) was dissolved in sterile physiological saline (0.9%), and administered to mice at the dose of 0.2 mg/4 μL/per mouse through i.c.v. route using a previously reported method by Haley and McCormick, 1957. Animals were euthanized using pentobarbital (60 mg/kg, i.p.) after administration of STZ. The treated animals (STZ groups) were tested 24 h after drug injection for behavioral or molecular assessments. In order to exclude the possible effect of i.c.v. saline injection, animals in the sham group were treated with sterile saline (4 μL/per mouse, i.c.v. route) and were tested 24 h after saline injection. Dose and the administration time of STZ were selected based on our pilot studies and previously published data (Souza et al., 2013b).

Experimental design

Animals were divided into two groups: Sham (treated by saline) and experimental (treated with STZ). After treatment with STZ (0.2 mg/4 μl/mouse), the animals were subjected to the behavioral tests which include OFT and HBT ($n = 6$), FST and Splash test ($n = 6$). For cellular and molecular experiments, different sets of animals were used in each group. Molecular evaluations include nitrite assay ($n = 6$), serum glucose level ($n = 10$), cytoplasmic phospholipase A2 (cPLA2) activity ($n = 5$), gene expression ($n = 4$), mitochondrial function ($n = 4$) and histopathological evaluation ($n = 3$). As reported by previous reports, the *in vitro* assays used in this study are highly reproducible and groups of 3–5 animals would suffice to obtain reliable results (Amiri et al., 2015a,b; Sonei et al., 2016).

BEHAVIORAL ASSESSMENTS

Forced swimming test (FST)

In this test, prolongation of immobility time in response to an inescapable challenge reflects the despair behavior in rodents (Porsolt et al., 1977). In brief, 24 h after the injection of saline or STZ, animals were placed in cylinders (10 × 25 cm, diameter × height) containing 19 cm of water at 23 ± 1 °C for 6 min. The immobility time was recorded during the last 4 min of the test by a blinded investigator. A mouse was considered to be immobile when it remained floating motionless in the water and made negligible movements to keep its head above water.

Splash test

In rodents, motivational and self-care difficulties can be assessed by splash test. In this test, we measured the grooming behavior of mice which is considered as an indirect measure of palatable solution intake. A 10% sucrose solution was squirted on the dorsal coat of

animals while they were in their home cages and mice were videotaped for 5 min. In this test, grooming activity behaviors include nose/face grooming, head washing, and body grooming (David et al., 2009; Detanico et al., 2009; Haj-Mirzaian et al., 2015).

Open-field test (OFT)

This test is used as a criterion for the evaluation of motor function and anxiety-like behaviors (Kulesskaya and Voikar, 2014). The OFT box was made of Plexiglas (50 cm × 50 cm × 40 cm), which was dimly illuminated during the test. Mice were placed individually on the corner of the box, and their behaviors were videotaped for 5 min and were analyzed by Ethovision software version 8 (Noldus, Netherlands). Following measures were assessed in this test; distance moved (horizontal activity), number of rearings (vertical activity), and time spent in the central zone (30 cm × 30 cm).

Hole-board test (HBT)

Hole-board test is a valid test to assess the anxiety-like behaviors in rodents (Takeda et al., 1998; Amiri et al., 2015b). The hole-board apparatus was made of Plexiglas (50 cm × 50 cm) with sixteen equally 3-cm diameter holes and was placed 50 cm above the floor. The apparatus was dimly illuminated (40 lx) and the number of head-dips of each mouse was counted in a 5-min period by an experimenter who was blind to treatment conditions. Reduction in the frequency of head-dips was considered as anxious behavior of animals.

Serum preparation

The mice were anesthetized (60 pentobarbital mg/kg, i.p.) to open the heart for the blood collection in test tube. Then, the test tube was put at 37 °C for 30 min to coagulate the blood. The serum was separated by centrifugation at 3,500 rpm for 10 min and stored at −80 °C until further analysis.

Tissue preparation

Animals were fasted overnight and then sacrificed. Hippocampi were dissected out and stored at −80 °C. The samples were divided into three different groups; first set of samples were used for preparation of tissue homogenate, on which measurement of oxidative stress parameters, the activity of phospholipase A_2 (PLA_2) and nitrite levels were performed. Second set of samples were used for total RNA extraction. The last set of samples were fixed in 10% formalin, sectioned, and stained with hematoxylin and eosin (H&E) for pathological evaluations.

Mitochondrial preparation

Animals (24 h after saline or STZ injections) were decapitated and hippocampi were dissected on ice, immersed in liquid nitrogen and were stored in −80 °C freezer. Homogenization was done at 4 °C using cold mannitol solution medium (Lores-Arnaiz et al., 2010). The homogenate was centrifuged at 1000*g* for 10 min at 4 °C. The supernatant was centrifuged at 10,000*g* for 10 min as a source of hippocampal mitochondria. The heavy mitochondrial fraction was collected and re-suspended in the mannitol solution and, re-centrifuged twice at 10,000g for 10 min. The resulting pellet (P_2 fraction), including both synaptic and non-synaptic mitochondria was re-suspended in desired buffer based on oxidative stress markers including ROS production, ATP, and glutathione (GSH).

ROS formation

The mitochondrial H_2O_2 production was assayed by flowcytometer via incubation of mitochondrial suspension with 2′,7′-dichlorofluorescein diacetate (DCFH-DA) (final concentration of 10 μM) in respiratory buffer (Gao et al., 2009) using the Flomax software (equipped with a 488-nm argon ion laser). DCFH-DA was used as reagent. Fluorescence signals were obtained using a 530-nm band pass filter (FL-1 channel) at least 12,000 counts per sample (Gao et al., 2009; Hosseini et al., 2014).

ATP levels

ATP level in each sample was measured by applying luciferase enzyme, and using Sirius tube luminometer (Berthold Detection System, Germany) as previously described in our lab (Hosseini et al., 2014).

Glutathione (GSH) levels

Glutathione levels were determined using 5,5′-dithiobis-(2-nitrobenzoic acid) or DTNB reagent. The developed yellow color was read at 412 nm using a spectrophotometer (UV-1601 PC, Shimadzu, Japan) and expressed as μg/mg protein based on calibration standard curve (Jayakumar et al., 2014).

Nitrite levels

Nitrite levels in the hippocampi were determined by the Griess method at 540 nm using $NaNO_2$ (Sigma, USA) for preparation of standard curve. Concentration of nitrite was normalized to the weight of each sample (Ding et al., 2010; Kordjazy et al., 2015).

Measurement the activity of $cPLA_2$

Phospholipase A_2 activity was measured by a $cPLA_2$ Assay Kit (Cayman chemical, Michigan, USA) and was reported as nano mol/min/mg protein (Nikoui et al., 2015).

Real time RT-PCR

Total RNA was extracted from hippocampi using TRIzol reagent (Invitrogen). Alterations in mRNA levels of selected genes were measured by qRT-PCR following reverse transcription of 1 μg of RNA from each sample using PrimeScript RT reagent kit (Takara Bio, Inc., Otsu, Japan). qRT-PCR was performed on a light cycler instrument (Roche Diagnostics, Mannheim, Germany)

using SYBR Premix Ex Taq technology (Takara Bio). Thermal cycling conditions consisted of an initial activation step for 30 s at 95 °C followed by 45 cycles including a denaturation step for 5 s at 95 °C and a combined annealing/extension step for 20 s at 60 °C. Melting curve analysis was applied to validate whether all primers yielded a single PCR product. The genes and their used primers are listed in Table 1. Hypoxanthine phosphoribosyl transferase1 (hprt1) was amplified as normalizer and the fold change in the expression of each target mRNA relative to hprt1 was calculated on the basis of $2^{-\Delta\Delta ct}$ relative expression formulas.

Microscopy

Animals ($n = 4$) were euthanized under anesthesia using pentobarbital (60 mg/kg,i.p.), 24 h after STZ injection. Trans-cardiac perfusion was performed via 0.9% normal saline first and then continued with ice-cold 4% paraformaldehyde in 0.1Mphosphate buffer (pH 7.5). Then, the brain was isolated. After fixation, the brain tissues were immersed in 10% formalin. Formalin-fixed brains were paraffin-embedded and 5 μm sections were obtained. Five sections obtained from each brain and were deparaffinized using xylene and stained with H&E. Histological analysis was performed under light microscopy (400; Olympus microscope) after preparing images under objective lens using a digital camera (Olympus, Japan) and displayed on a computer monitor. Three fields from each slide were selected and the density of dark neurons and normal neurons within the pyramidal cell layer of both CA1 and CA3 areas was estimated in each field. In histological studies dark neurons are recognized by hyperbasophilia property as a type of cell degeneration (Zsombok et al., 2005). The relation of normal neurons to normal neurons + dark neurons (total number of neurons) was evaluated in each group. The fields were randomly selected. Moreover, the maximum and minimum nucleus diameter was measured and the average of the measurements was reported for each group. The thickness of pyramidal cell layer of CA1 and CA3 areas was measured. The linear measurements were performed at determinate points along the CA_1 and CA_3 subfields. All measurements were performed using Image J software by a blinded pathologist.

Glucose levels

Serum glucose concentrations were measured either before the injection of STZ or different time intervals after STZ (i.c.v.) injections (6 h, 24 h, and 48 h). Each animal was decapitated under mild anesthesia using pentobarbital (60 mg/kg, i.p.) and the blood was collected. Serum glucose concentrations were measured by the glucose oxidase method (Glucose Analyzer II, Beck-man).

Protein assay

We used Coomassie blue protein-binding method by using BSA as the standard for measuring the mitochondrial protein levels (Bradford, 1976). To keep the uniformity of experimental condition, the mitochondrial samples (100 μg /ml mitochondrial protein) were used in all experiments.

Statistics

The sample size was calculated by power calculations using G power software (ver.3.1.7, Franz Faul, Universitat Kiel, Germany). We set α error at 0.05 and power (1-β) at 0.8 and the required total sample size per group was calculated as 6–8 in behavioral tests and 3–6 in molecular studies. Comparison between the groups was analyzed using *t*-test and a one-way analysis of variance (ANOVA) followed by tukey's post hoc tests using the Graph-pad prism software (version 6). $P < 0.05$ was considered statistically significant.

RESULTS

Administration of STZ provoked behaviors associated with depression and anxiety in mice

Analyses revealed that i.c.v. administration of STZ provoked behaviors relevant to depression and anxiety in male adult mice. We demonstrated the impact of STZ injection on different aspects of depressive-like behaviors such as despair behavior and deficit in motivation using FST and splash test. The *t*-test analysis results showed that the immobility time was increased in STZ-treated animals when compared with sham group in the FST ($t = 6.152$, $df = 10$, $P < 0.001$, Fig. 1a). Also, STZ treatment caused a significant reduction in grooming activity time in the splash test

Table 1. Primers

Gene	Sequence (5′ → 3′)	
	Forward	Reverse
Il-6	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTTGG
Il-1β	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG
TNF-α	CTGAACTTCGGGGTGATCGG	GGCTTGTCACTCGAATTTTGAGA
Hprt1	TGCTCGAGATGTGATGAAGG	AAGCAGATGGCCACAGAACT
Myd88	ATCGCTGTTCTTGAACCCTCG	CTCACGGTCTAACAAGGCCAG
Tlr-2	CTCTTCAGCAAACGCTGTTCT	GGCGTCTCCCTCTATTGTATTG
Tlr-4	ATGGCATGGCTTACACCACC	GAGGCCAATTTTGTCTCCACA
Nlrp3	ATCAACAGGCGAGACCTCTG	GTCCTCCTGGCATACCATAGA

when compared with the sham group (t = 5882, df = 10, P < 0.001, Fig. 1b).

The possible effect of STZ injection on locomotor activity and anxiety-like behaviors was determined using OFT and HBT. In the OFT, t-test analyses revealed that there is no significant difference in the total distance moved (horizontal activity) (t = 0.1117, df = 10, P > 0.05, Fig. 1c) and number of rearings (vertical activity) (t = 0.0571, df = 10, P > 0.05, Fig. 1d) between treated groups. As shown in Fig. 1e, f, analyses demonstrated that there were significant differences in time spent in the central zone in OFT (t = 5.320, df = 10, P < 0.001, Fig. 1e) and number of head-dips in the HBT (t = 4.503, df = 10, P < 0.001, Fig. 1f) between sham and STZ-treated groups. Also, we observed that STZ-treated animals exhibited behavioral abnormalities for more than 21 days after STZ injection.

Serum glucose level

In comparison with sham groups, one-way ANOVA analysis followed by tukey's post test revealed that there was no significant difference in serum glucose level between 6, 24 and 48 h time intervals after STZ administration (Data not Shown).

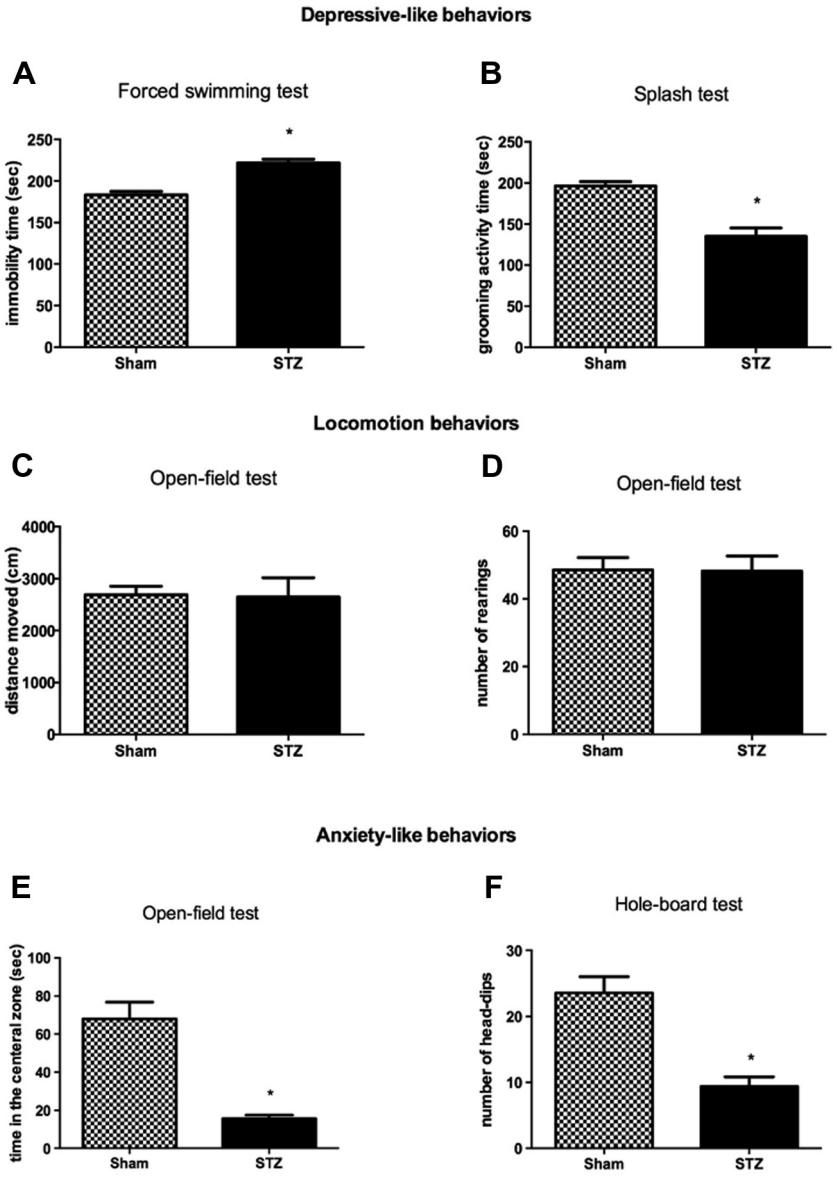

Fig. 1. Effect of intracerebroventricular STZ administration on behaviors related to depression and anxiety and locomotor activity. Effect of STZ (0.2 mg/4 μL/mouse) (STZ group) and saline (4 μL/mouse) (Sham group) intracerebroventricular injections on the immobility time in the FST (A), grooming activity time in the splash test (B), total distance moved in the OFT (C), number of rearings in the OFT (D), time spent in the central zone in the OFT (E), and number of head-dips in the HBT (F). Values are expressed as mean ± S.E.M. and were analyzed using t-test. $^{*}P$ < 0.05 compared with the sham group in each figure.

STZ increased cPLA2 activity and increased the expression of genes relevant to immune-inflammatory system in the hippocampus

Fig. 3 shows the effects of STZ on the genes relevant to immune-inflammatory pathways. In comparison with the sham group, t-test analysis demonstrated the up-regulation of *Tlr-2* (t = 3.150, df = 6, P < 0.05), *Tlr-4* (t = 2.642, df = 6, P < 0.05), *Myd88* (t = 2.713, df = 6, P < 0.05), *Il-6* (t = 4.176, df = 6, P < 0.01), and *Nlrp-3* (t = 10.55, df = 6, P < 0.001) in the hippocampus of STZ-treated mice. In addition, we observed no significant differences in the expression of *Il-1ß* (t = 0.1639, df = 6, P > 0.05) and *Tnf-α* (t = 0.1414, df = 6, P > 0.05) in STZ-treated mice when compared with sham mice. In addition, there was no significant effect of treatments (STZ vs. saline) on *Hprt1* expression.

Further, our results showed a significant effect of STZ treatment on $cPLA_2$ activity in the hippocampus. In this regard, *t-test* analysis revealed that there was a significant difference in $cPLA_2$ activity between saline and STZ-treated groups (t = 7.753, df = 8, P < 0.001, Fig. 4). Further, as shown in Fig. 4, tukey's analyses revealed that $cPLA_2$ had a higher activity in the hippocampal tissue of STZ-treated animals when compared with sham mice (P < 0.001).

STZ affected hippocampal mitochondria function and induced nitrosative stress

The effects of STZ treatment on hippocampal mitochondrial GSH, ATP and nitrite levels are presented in Table 2. Results obtained from a

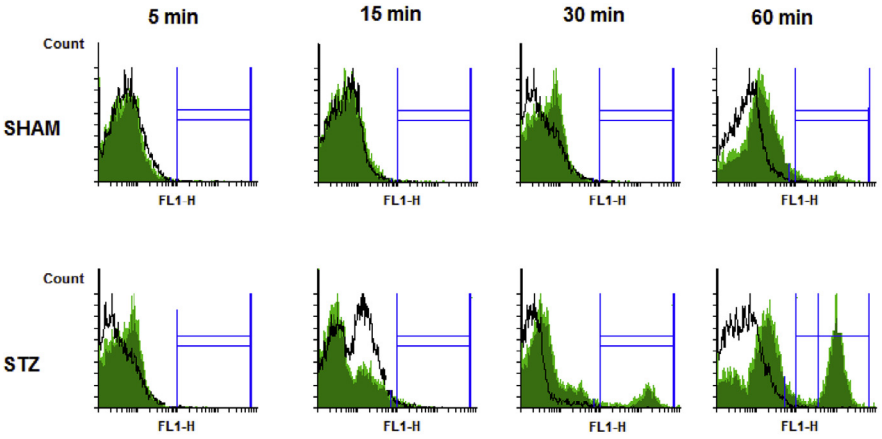

Fig. 2. Effects of intracerebroventricular STZ administration on ROS formation in the hippocampus. The ROS in each sample was read with 485 nm excitation and 520 nm emission using a fluorimeter after 5, 15, 30, and 60 min in Sham (received saline i.c.v.) and STZ treated (STZ group) animals. The signs for increased ROS formation in flowcytograms are shifting the ROS peak to the right and increasing of AUC.

one-way ANOVA analysis revealed that there was a significant difference in GSH, ATP, and nitrite levels between groups. Further, tukey's analyses revealed significant decrease in GSH ($P < 0.001$), ATP ($P < 0.001$), and nitrite levels ($P < 0.001$) in STZ-treated mice as compared to sham groups.

Moreover, the assessment of ROS formation was performed in 4 time intervals (5 min, 15 min, 30 min, and 60 min) in STZ-treated and sham groups (Fig. 2). Increased mitochondrial ROS formation in flowcytograms is presented as shifting the ROS peak to the rightward not just increased AUC. As shown in Fig. 2, in comparison to sham, there is a significant rightward shift of DCF peak (concentration dependent) in the hippocampus of STZ-treated group

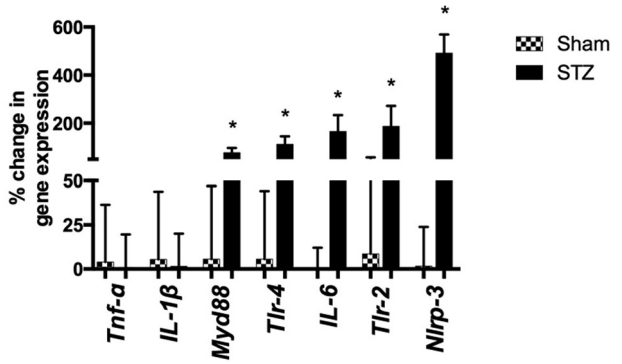

Fig. 3. Effect of intracerebroventricular STZ administration on hippocampal immune-inflammatory markers. Effect of intracerebroventricular saline (Sham group) and STZ (STZ group) injections on *Tnf-α*, *IL-1β*, *Il-6*, *Nlrp-3*, *Tlr-4, Tlr-2* and *Myd88* gene expressions in the hippocampus of the animals, Values are expressed as mean ± S.E.M. and were analyzed using *t*-test. $^{*}P < 0.05$ compared with the sham group in each coupled columns.

STZ did not induce hippocampal damage in histopathological studies

Our histopathological evaluation after the experimental period showed no significant difference between sham and STZ treated groups in the number of neurons at the CA_1 and CA_3 panels of hippocampus. The results of STZ injection in the CA_1 panel of hippocampus showed some reduction in neuronal density (neuronal drop-out), although this difference was not significant. Twenty-four hours following STZ injection, we observed no significant differences between the groups in the normal/all pyramidal neurons ratios in the CA_1 and CA_3 areas as well as diameters of the CA_1 and CA_3 areas of hippocampus ($P > 0.05$, Fig. 5). Moreover, there was significant difference in the nuclear diameter of pyramidal cells in the CA_1 and CA_3 area between sham and STZ groups ($P < 0.05$, Fig. 5).

DISCUSSION

In the current study, we found that negative affective behaviors following the administration of STZ

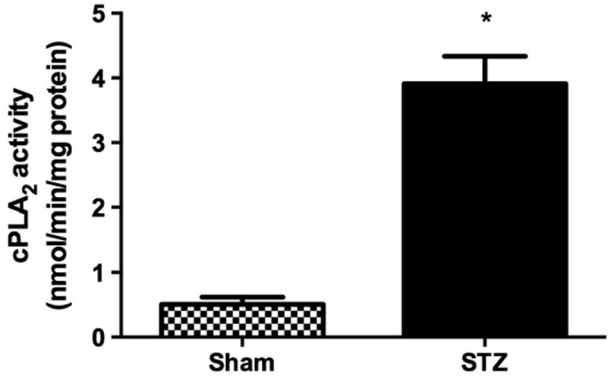

Fig. 4. Effect of intracerebroventricular STZ administration on hippocampal cPLA2 activity. Effect of intracerebroventricular saline (Sham group) and STZ (STZ group) injections on cPLA2 activity (nmol/min/mg protein), Values are expressed as mean ± S.E.M. and were analyzed using *t*-test. $^{*}P < 0.001$ compared with the sham group.

Table 2. Effect of intracerebroventricular STZ administration (0.2 mg/mice) on GSH, ATP, and nitrite level in the hippocampus: Values are expressed as Mean ± S.D. and were analyzed using one-way ANOVA followed by tukey's post hoc tests $^{*}P < 0.001$ compared with Sham group

	μg GSH/mg protein	nmol ATP/mg protein	nmol Nitrite/mg tissue
Sham	13.9 ± 3.4	2.12 ± 0.23	81.3 ± 5
STZ	4.47 ± 1.9*	1.39 ± 0.16*	129.6 ± 4.6*

(0.2 mg/mouse, i.c.v.) are associated with impairment in the mitochondrial function, oxidative challenge, and immune-inflammatory responses in the hippocampus of mice.

Using rodent models of STZ-induced diabetes/AD, several investigations have shown that depressive behaviors in STZ-treated rodents are associated with oxidative challenge and neurochemical changes in the hippocampus (Haider et al., 2013; de Morais et al., 2014; Lee et al., 2015). By using FST, as a cogent behavioral test for the evaluation of passive behaviors in mice, we observed a significant increase in the immobility time of STZ-treated mice after 24 h. The increase in immobility time reflects the behavioral despair in humans as a core symptom of depression (Cryan and Holmes, 2005). In addition, the results of splash test indicate the presence of motivational and self-care difficulties in animals 24 h following STZ administration. The decrease in grooming activity time in response to 10% sucrose is considered as a behavioral measure for assessing motivation and self-care deficits in both mice and rats (David et al., 2009; Marrocco et al., 2014). Furthermore, STZ produced anxiety-like behaviors in mice subjected to HBT and OFT. In this context, STZ-treated mice exhibited a remarkable decline in the number of head-dips in HBT, and avoidance to enter the central zone of OFT. This behavioral profile suggests that STZ is able to induce anxiety-like behaviors in mice as the most prevalent comorbid condition observed in depressed patients. In this context, our findings are in agreement with those studies which have reported that behavioral abnormalities in mice treated with STZ (i.c.v.) are associated with increased inflammation in the brain (Souza et al., 2013b; Ho et al., 2014).

Clinical and preclinical studies indicate that depression either as a comorbid condition in a systemic disease or as a result of exposure to chronic stress correlates with inflammation (Slavich and Irwin, 2014). It has been evident that depression can occur following specific medical conditions such as stroke, stimulants withdrawal, PTSD, and traumatic brain injury (Barr et al., 2002; Caeiro et al., 2006; Gill et al., 2009). In addition, single-dose administration of LPS is known to produce depressive-like behaviors in animals after 24 h, and it is used as a valid animal model of depression (Souza et al., 2013a). Furthermore, besides pathogen-induced inflammation, sterile inflammation has also been involved in the development of mood disorders (Anisman, 2009; Walker, 2013). Similar to LPS studies, we used STZ to investigate the role of sterile inflammation (versus microbial-dependent inflammation) 24 h following STZ administration. It is important to note that animals in our study were under anesthesia during administration of STZ and they experienced no acute stress.

Emerging lines of evidence suggests that mitochondrial dysfunction is the triggering factor for the development of the majority of brain disorders such as depression (Gardner and Boles, 2011; Morava and Kozicz, 2013). In this context, evidence is accumulating to show that STZ is able to induce mitochondrial dysfunction, energy challenge, and inflammatory responses in the brain (Chen et al., 2013; Rajasekar et al., 2014). Our results revealed that energy hemostasis and redox state dramatically underwent negative changes in the hippocampus of STZ-treated mice. A massive production of ROS and NO, and decreased levels of ATP and GSH present a picture in which energy metabolism and antioxidant system undergo negative changes in the hippocampus of mice 24 h after STZ treatment. Previous studies have demonstrated that mitochondrial ROS contributes to cellular damage, and these deleterious effects are more severe when GSH (the main antioxidant of the brain) depletion occurs (Hosseini et al., 2014; Gawryluk et al., 2011). Additionally, the increased levels of nitrite in the hippocampus of STZ-treated mice suggest the involvement of NO overproduction in the initiation of sterile inflammation. Under pathologic conditions, inducible nitric oxide synthase (iNOS) produces NO which not only augments the injurious effects of ROS through the formation of peroxynitrite radicals, but harmfully affects mitochondrial function and energy metabolism (Brown, 2001; Liu et al., 2002). In addition, nitrergic system plays an important part in the pathobiology of psychiatric disorders such as anxiety and depression (Chen et al., 2015; Amiri et al., 2015a). In the current study, we found a considerable increase in $cPLA_2$ activity in the hippocampus of STZ-treated mice. The increased activity of $cPLA_2$ triggers several inflammatory signaling cascades such as cyclooxygenase and lipoxygenase pathways and harms intracellular structures such as lysosomes. Since $cPLA_2$ is a calcium-dependent enzyme, the increased activity of $cPLA_2$ in the hippocampus not only confirms the elevated levels of Ca^{2+}, but also corroborates the activation of inflammatory signaling in the hippocampus of STZ-treated mice. In this regard, previous studies have shown that $cPLA_2$ mediates a variety of oxidative and inflammatory pathways in the brain following acute exposure to immune challenge, and adversely affects animal behaviors (Sun et al., 2010; Hermann et al., 2013). Finally, all these molecular changes result in energy and redox challenge in the cells which consequently lead to the formation of cellular injury and stress (Festjens et al., 2006).

Recent evidence suggests that under sterile inflammatory conditions, DAMPs and ROS are key triggers for innate immunity responses (Kang et al., 2014; Choi et al., 2015). In this regard, DAMPs have been demonstrated as endogenous ligands for the activation of TLRs (Fleshner, 2013; Lucas and Maes, 2013). Once activated, TLRs (mainly TLR-2 and TLR-4) initiate several intracellular signaling pathways which are associated with the upregulation of inflammatory factors such as NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) MyD88, IL-6, and iNOS. On the other hand, mitochondrial-derived ROS not only engages in DAMPs production through oxidizing intracellular components, but is able to activate NLRP3 inflammasome formation (Chen and Nuñez, 2010a,b; West et al., 2011). Our results demonstrated that STZ is able to induce a significant increase in *Myd88* (main player in sterile inflammation) as well as its upstream (*Tlr-2* and *Tlr-4* as the main TLRs in sterile inflammation) and downstream (*Il-6*) expression (Kono et al., 2014). In addition, the overexpression of *Nlrp3* and other innate-immunity compo-

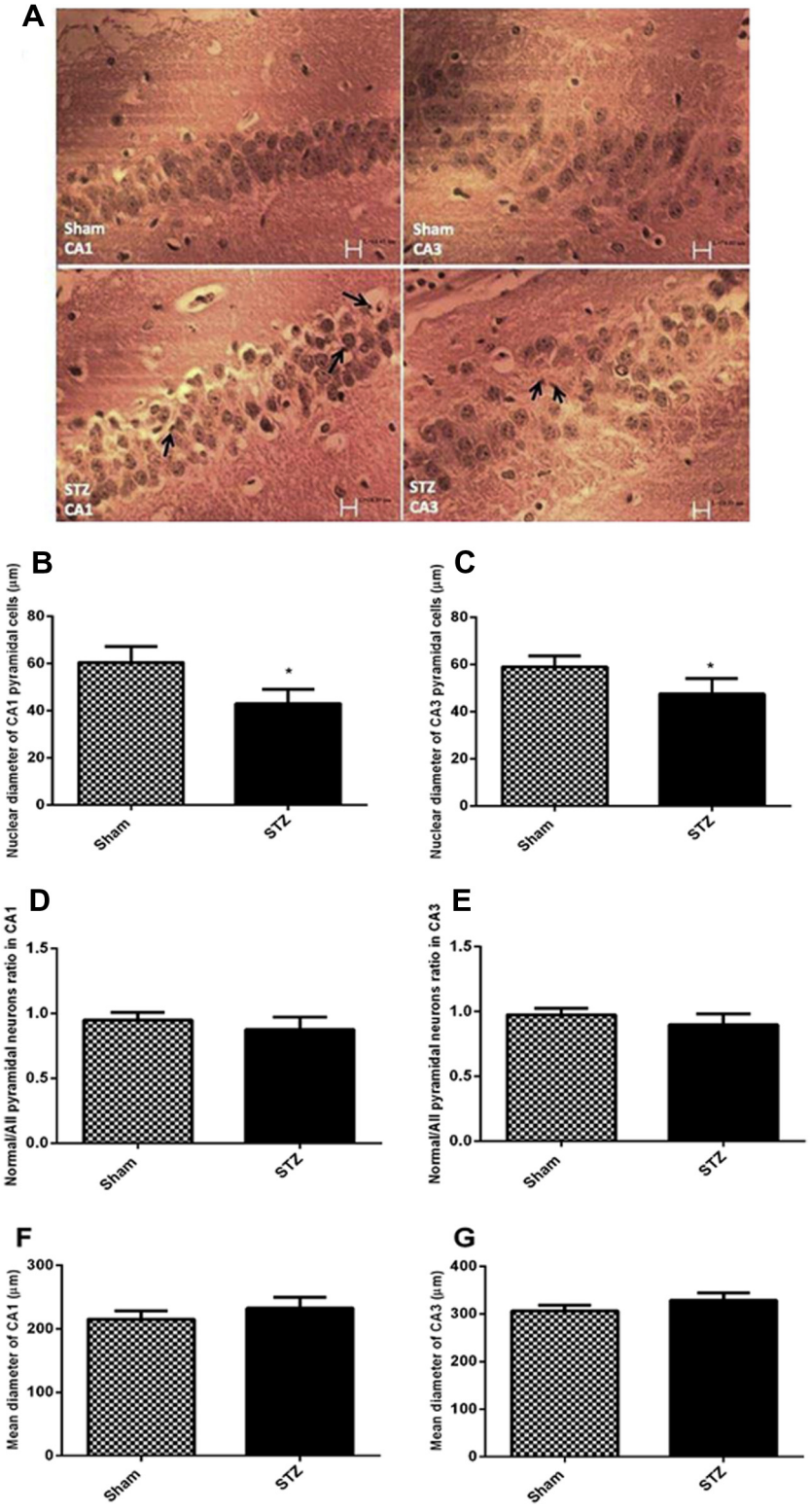

Fig. 5. The effects of STZ injection on hippocampal CA1 and CA3 areas 24 h after injection in NMRI mice, (A): Representative hematoxylin and eosin (H&E) stained slides from CA1 and CA3 areas (×400). (B and C): Nuclear diameter of pyramidal neurons in the CA1 and CA3 areas. (D and E): Normal/all pyramidal neurons ratio CA1 and CA3 areas. (F and G): Mean diameter of the CA1 and CA3 areas between sham and STZ groups.

nents following STZ administration suggest the possible role of these factors in the development of the affective-like behaviors through sterile inflammation. Further, these results indicate that mitochondrial dysfunction and oxidative challenge are associated with initiation of inflammatory responses following STZ treatment. Interestingly, we observed no alteration in the mRNA expression of *Il-1β* 24 h following STZ injection. To explain the latter observation, a recent study on thromboembolic stroke in mice has shown that the activation of IL-1β occurs 24 h after the insult (Abulafia et al., 2009). The expression and activation of IL-1β is highly dependent to a variety of factors such as severity, nature and duration of DAMPs exposure. For example, the cleavage of pro-IL-1β by caspase-1 does not occur without the primary stimulation of microglia (Brough et al., 2011; Kono et al., 2014). In comparison with thromboembolic stroke, we induced a comparatively less severe sterile inflammation in our study. Interestingly, the results of histological assessment revealed no sign of neurodegeneration in the hippocampus of STZ-treated mice indicating that affective-like behaviors of animals were not associated with neurodegeneration. Using STZ-induced dementia, a recent study by Kraska et al. revealed that low dose STZ (1 mg, icv) induces moderate inflammation and neuronal loss after 3 months (Kraska et al., 2012). In this study, we applied a comparatively low dose of STZ (0.2 mg, icv) to mice and also, we evaluated the STZ-induced effects 24 h following treatments. Thus, unchanged expression of *Il-1β* may be associated with the low intensity of the insult by the low dose STZ.

Examples of such conditions are behavioral abnormalities following an ischemic challenge such as stroke and traumatic brain injury. Interestingly, recent evidence indicates that inflammasomes and mitochondrial dysfunction are of main etiological factors for the development of metabolic disorders and their behavioral comorbidities (Choi and Ryter, 2014; Peeri and Amiri, 2015). Since STZ has been long used in the modeling of experi-

mental diabetes and AD and recently depression in animals, our results suggest that similar mechanisms may play a part in the pathophysiology of other metabolic disorders such as diabetes and AD. In addition, one of the limitations of this study is that we did not measure the activity of stress axis. Dysfunction of hypothalamic–pituitary–adrenal (HPA) axis is known as the main contributor in the pathophysiology of depression, and chronic exposure to glucocorticoids leads to the development of depression. However, even if HPA axis had a severe response to STZ challenge, acute increase in glucocorticoids would not be the main factor responsible for the emergence of anxiety and mood disorders in animals. It is important to note that the acute effects of glucocorticoids are not associated with depressive and anxiety-like behaviors and only chronic exposure to glucocorticoids has been reported as a risk factor for the development of depression (McEwen, 2004, 2005). In addition, an interesting clinical study by Miller et al. has revealed that depressive episodes following acute stress are associated with inflammatory factors and not glucocorticoids (Miller et al., 2005). Another limitation of this study is that we did not evaluate the effects of STZ on female mice. In our future studies, we decide to evaluate the effects of STZ in female mice to see how hormonal changes during different cycles of estrus cycle may alter the behavioral and molecular responses of animals. Finally, this procedure could be used as an animal model for sterile inflammation-induced behavioral abnormalities. Such conditions are observed following traumatic brain injury and stroke. However, it is now well determined that corticosteroids and chronic stress are key factors which contribute to the pathophysiology of depression. Thus, this animal model is not an appropriate model for evaluating the pathophysiology of depression after exposure to social/environmental stressors.

Furthermore, although STZ is not able to transfer blood brain barrier, we also measured the blood glucose levels in order to show that STZ has no effect on peripheral metabolism state after 24 h. We also evaluated the effects of acute administration of fluoxetine and aminoguanidine (iNOS inhibitor) 24 h following STZ treatment. We observed that these treatments could effectively reverse the behavioral abnormalities and mitochondrial dysfunction in the hippocampus of mice indicating that inflammatory responses are involved in the emergence of behavioral abnormalities following sterile inflammation (data not shown).

CONCLUSION

Overall, results of this work provided evidence that affective-like behaviors in mice following single administration of low dose STZ into lateral ventricles are associated with negative changes in mitochondrial function and inflammatory status in the hippocampus, and these factors at least in part are associated with the appearance of behavioral deficits 24 h following STZ treatment.

CONFLICT OF INTEREST

None declared.

Acknowledgment—This work was supported by grants from the deputy of research of Zanjan University of Medical Sciences (Grant no: A-12-769-12) and research grant from Tehran University of Medical Sciences, Tehran, Iran (Grant no: 24383).

REFERENCES

Abulafia DP, de Rivero Vaccari JP, Lozano JD, Lotocki G, Keane RW, Dietrich WD (2009) Inhibition of the inflammasome complex reduces the inflammatory response after thromboembolic stroke in mice. J Cereb Blood Flow Metab 29:534–544.

Amiri S, Amini-Khoei H, Haj-Mirzaian A, Rahimi-Balaei M, Naserzadeh P, Dehpour A, Mehr SE, Hosseini M-J (2015a) Tropisetron attenuated the anxiogenic effects of social isolation by modulating nitrergic system and mitochondrial function. Biochim Biophys Acta 1850:2464–2475.

Amiri S, Haj-Mirzaian A, Rahimi-Balaei M, Razmi A, Kordjazy N, Shirzadian A, Mehr SE, Sianati H, Dehpour AR (2015b) Co-occurrence of anxiety and depressive-like behaviors following adolescent social isolation in male mice; possible role of nitrergic system. Physiol Behav 145:38–44.

Anisman H (2009) Cascading effects of stressors and inflammatory immune system activation: implications for major depressive disorder. J Psychiatry Neurosci 34:4.

Barr AM, Markou A, Phillips AG (2002) A 'crash' course on psychostimulant withdrawal as a model of depression. Trends Pharmacol Sci 23:475–482.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254.

Brough D, Tyrrell PJ, Allan SM (2011) Regulation of interleukin-1 in acute brain injury. Trends Pharmacol Sci 32:617–622.

Brown GC (2001) Regulation of mitochondrial respiration by nitric oxide inhibition of cytochrome c oxidase. Biochim Biophys Acta 1504:46–57.

Caeiro L, Ferro JM, Santos CO, Figueira ML (2006) Depression in acute stroke. J Psychiatry Neurosci 31:377.

Chen GY, Nuñez G (2010a) Sterile inflammation: sensing and reacting to damage. Nat Rev Immunol 10:826–837.

Chen H-JC, Spiers JG, Sernia C, Lavidis NA (2015) Response of the nitrergic system to activation of the neuroendocrine stress axis. Front Neurosci 9.

Chen Y, Liang Z, Blanchard J, Dai C-L, Sun S, Lee MH, Grundke-Iqbal I, Iqbal K, Liu F, Gong C-X (2013) A non-transgenic mouse model (icv-STZ mouse) of Alzheimer's disease: similarities to and differences from the transgenic model (3xTg-AD mouse). Mol Neurobiol 47:711–725.

Chen GY, Nuñez G (2010b) Sterile inflammation: sensing and reacting to damage. Nat Rev 10:826–837.

Choi AJ, Ryter SW (2014) Inflammasomes: molecular regulation and implications for metabolic and cognitive diseases. Mol Cells 37:441.

Choi H-S, Kang J-W, Lee S-M (2015) Melatonin attenuates carbon tetrachloride–induced liver fibrosis via inhibition of necroptosis. Transl Res.

Cryan JF, Holmes A (2005) The ascent of mouse: advances in modelling human depression and anxiety. Nat Rev Drug Discovery 4:775–790.

David DJ, Samuels BA, Rainer Q, Wang J-W, Marsteller D, Mendez I, Drew M, Craig DA, Guiard BP, Guilloux J-P (2009) Neurogenesis-dependent and-independent effects of fluoxetine in an animal model of anxiety/depression. Neuron 62:479–493.

de Morais H, de Souza CP, da Silva LM, Ferreira DM, Werner MF, Andreatini R, da Cunha JM, Zanoveli JM (2014) Increased oxidative stress in prefrontal cortex and hippocampus is related

to depressive-like behavior in streptozotocin-diabetic rats. Behav Brain Res 258:52–64.
Detanico BC, Piato ÂL, Freitas JJ, Lhullier FL, Hidalgo MP, Caumo W, Elisabetsky E (2009) Antidepressant-like effects of melatonin in the mouse chronic mild stress model. Eur J Pharmacol 607:121–125.
Ding J, Li QY, Wang X, Sun CH, Lu CZ, Xiao BG (2010) Fasudil protects hippocampal neurons against hypoxia-reoxygenation injury by suppressing microglial inflammatory responses in mice. J Neurochem 114:1619–1629.
Festjens N, Berghe TV, Vandenabeele P (2006) Necrosis, a well-orchestrated form of cell demise: signalling cascades, important mediators and concomitant immune response. Biochim Biophys Acta 1757:1371–1387.
Fleshner M (2013) Stress-evoked sterile inflammation, danger associated molecular patterns (DAMPs), microbial associated molecular patterns (MAMPs) and the inflammasome. Brain Behav Immun 27:1–7.
Fournier JC, DeRubeis RJ, Hollon SD, Dimidjian S, Amsterdam JD, Shelton RC, Fawcett J (2010) Antidepressant drug effects and depression severity: a patient-level meta-analysis. JAMA 303:47–53.
Gao P, Qian DH, Li W, Huang L (2009) NPRA-mediated suppression of AngII-induced ROS production contribute to the antiproliferative effects of B-type natriuretic peptide in VSMC. Mol Cell Biochem 324:165–172.
Gardner A, Boles RG (2011) Beyond the serotonin hypothesis: mitochondria, inflammation and neurodegeneration in major depression and affective spectrum disorders. Prog Neuropsychopharmacol Biol Psychiatry 35:730–743.
Gawryluk JW, Wang J-F, Andreazza AC, Shao L, Young LT (2011) Decreased levels of glutathione, the major brain antioxidant, in post-mortem prefrontal cortex from patients with psychiatric disorders. Int J Neuropsychopharmacol 14:123–130.
Gill JM, Saligan L, Woods S, Page G (2009) PTSD is associated with an excess of inflammatory immune activities. Perspect Psychiatr Care 45:262–277.
Gurung P, Lukens JR, Kanneganti T-D (2015) Mitochondria: diversity in the regulation of the NLRP3 inflammasome. Trends Mol Med 21:193–201.
Haider S, Ahmed S, Tabassum S, Memon Z, Ikram M, Haleem DJ (2013) Streptozotocin-induced insulin deficiency leads to development of behavioral deficits in rats. Acta Neurol Belg 113:35–41.
Haj-Mirzaian A, Amiri S, Kordjazy N, Rahimi-Balaei M, Haj-Mirzaian A, Marzban H, Dehpour AR, Mehr SE (2015) Blockade of NMDA receptors reverses the depressant, but not anxiogenic effect of adolescence social isolation in mice. Eur J Pharmacol.
Haley TJ, McCormick WG (1957) Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. Br J Pharmacol Chemother 12:12–15.
Hermann PM, Park D, Beaulieu E, Wildering WC (2013) Evidence for inflammation-mediated memory dysfunction in gastropods: putative PLA2 and COX inhibitors abolish long-term memory failure induced by systemic immune challenges. BMC Neurosci 14:83.
Ho N, Brookshire BR, Clark JE, Lucki I (2014) Indomethacin reverses decreased hippocampal cell proliferation in streptozotocin-induced diabetic mice. Metab Brain Dis 30:555–562.
Hosseini M-J, Shaki F, Ghazi-Khansari M, Pourahmad J (2014) Toxicity of copper on isolated liver mitochondria: impairment at complexes I, II, and IV leads to increased ROS production. Cell Biochem Biophys 70:367–381.
Jayakumar S, Kunwar A, Sandur SK, Pandey BN, Chaubey RC (2014) Differential response of DU145 and PC3 prostate cancer cells to ionizing radiation: role of reactive oxygen species, GSH and Nrf2 in radiosensitivity. Biochim Biophys Acta 1840:485–494.
Kang R, Lotze MT, Zeh HJ, Billiar TR, Tang D (2014) Cell death and DAMPs in acute pancreatitis. Mol Med 20:466.
Kono H, Onda A, Yanagida T (2014) Molecular determinants of sterile inflammation. Curr Opin Immunol 26:147–156.
Kordjazy N, Haj-Mirzaian A, Amiri S, Ostadhadi S, Kordjazy M, Sharifzadeh M, Dehpour AR (2015) Elevated level of nitric oxide mediates the anti-depressant effect of rubidium chloride in mice. Eur J Pharmacol.
Kraska A, Santin MD, Dorieux O, Joseph-Mathurin N, Bourrin E, Petit F, Jan C, Chaigneau M, Hantraye P, Lestage P (2012) In vivo cross-sectional characterization of cerebral alterations induced by intracerebroventricular administration of streptozotocin. PLoS One 7(9) e46196.
Kulesskaya N, Voikar V (2014) Assessment of mouse anxiety-like behavior in the light-dark box and open-field arena: role of equipment and procedure. Physiol Behav 133:30–38.
Lee SG, Yoo DY, Jung HY, Nam SM, Kim JW, Choi JH, Yi SS, Won M-H, Yoon YS, Hwang IK (2015) Neurons in the hippocampal CA1 region, but not the dentate gyrus, are susceptible to oxidative stress in rats with streptozotocin-induced type 1 diabetes. Neural Regen Res 10:451.
Liu B, Gao HM, Wang JY, Jeohn GH, Cooper CL, Hong JS (2002) Role of nitric oxide in inflammation-mediated neurodegeneration. Ann N Y Acad Sci 962:318–331.
Lores-Arnaiz S, Lores Arnaiz MR, Czerniczynie A, Cuello M, Bustamante J (2010) Mitochondrial function and nitric oxide production in hippocampus and cerebral cortex of rats exposed to enriched environment. Brain Res 1319:44–53.
Lucas K, Maes M (2013) Role of the Toll Like receptor (TLR) radical cycle in chronic inflammation: possible treatments targeting the TLR4 pathway. Mol Neurobiol 48:190–204.
Maes M (2011) Depression is an inflammatory disease, but cell-mediated immune activation is the key component of depression. Prog Neuropsychopharmacol Biol Psychiatry 35:664–675.
Marrocco J, Reynaert M-L, Gatta E, Gabriel C, Mocaër E, Di Prisco S, Merega E, Pittaluga A, Nicoletti F, Maccari S (2014) The effects of antidepressant treatment in prenatally stressed rats support the glutamatergic hypothesis of stress-related disorders. J Neurosci 34:2015–2024.
McEwen BS (2004) Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. Ann N Y Acad Sci 1032:1–7.
McEwen BS (2005) Glucocorticoids, depression, and mood disorders: structural remodeling in the brain. Metabolism 54:20–23.
Miller GE, Rohleder N, Stetler C, Kirschbaum C (2005) Clinical depression and regulation of the inflammatory response during acute stress. Psychosom Med 67:679–687.
Morava É, Kozicz T (2013) Mitochondria and the economy of stress (mal) adaptation. Neurosci Biobehav Rev 37:668–680.
Nikoui V, Mehr SE, Jazaeri F, Ostadhadi S, Eftekhari G, Dehpour A-R, Mani AR, Bakhtiarian A (2015) Prostaglandin F 2α modulates atrial chronotropic hyporesponsiveness to cholinergic stimulation in endotoxemic rats. Eur J Pharmacol 748:149–156.
Peeri M, Amiri S (2015) Protective effects of exercise in metabolic disorders are mediated by inhibition of mitochondrial-derived sterile inflammation. Med Hypotheses 85:707–709.
Porsolt RD, Bertin A, Jalfre M (1977) Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 229:327–336.
Rai S, Kamat PK, Nath C, Shukla R (2014) Glial activation and post-synaptic neurotoxicity: the key events in Streptozotocin (ICV) induced memory impairment in rats. Pharmacol Biochem Behav 117:104–117.
Rajasekar N, Dwivedi S, Nath C, Hanif K, Shukla R (2014) Protection of streptozotocin induced insulin receptor dysfunction, neuroinflammation and amyloidogenesis in astrocytes by insulin. Neuropharmacology 86:337–352.
Reichenberg A, Yirmiya R, Schuld A, Kraus T, Haack M, Morag A, Pollmächer T (2001) Cytokine-associated emotional and cognitive disturbances in humans. Arch Gen Psychiatry 58:445–452.
Slavich GM, Irwin MR (2014) From stress to inflammation and major depressive disorder: A social signal transduction theory of depression. Psychol Bull 140:774.

Sonei N, Amiri S, Jafarian I, Anoush M, Rahimi-Balaei M, Bergen H, Haj-Mirzaian A, Hosseini M-J (2016) Mitochondrial dysfunction bridges negative affective disorders and cardiomyopathy in socially isolated rats: Pros and cons of fluoxetine. World J Biol Psychiatry:1–15.

Souza LC, Carlos Filho B, Fabbro LD, de Gomes MG, Goes AT, Jesse CR (2013a) Depressive-like behaviour induced by an intracerebroventricular injection of streptozotocin in mice: the protective effect of fluoxetine, antitumour necrosis factor-α and thalidomide therapies. Behav Pharmacol 24:79–86.

Souza LC, Carlos Filho B, Fabbro LD, de Gomes MG, Goes ATR, Jesse CR (2013b) Depressive-like behaviour induced by an intracerebroventricular injection of streptozotocin in mice: the protective effect of fluoxetine, antitumour necrosis factor-α and thalidomide therapies. Behav Pharmacol 24:79–86.

Sun GY, Shelat PB, Jensen MB, He Y, Sun AY, Simonyi A (2010) Phospholipases A2 and inflammatory responses in the central nervous system. Neuromolecular Med 12:133–148.

Takeda H, Tsuji M, Matsumiya T (1998) Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. Eur J Pharmacol 350:21–29.

Ustun TB (2001) The worldwide burden of depression in the 21st century. Treatment of depression: Bridging the 21st century 35–45.

Walker FR (2013) A critical review of the mechanism of action for the selective serotonin reuptake inhibitors: do these drugs possess anti-inflammatory properties and how relevant is this in the treatment of depression? Neuropharmacology 67:304–317.

West AP, Shadel GS, Ghosh S (2011) Mitochondria in innate immune responses. Nat Rev Immunol 11:389–402.

Yirmiya R (1996) Endotoxin produces a depressive-like episode in rats. Brain Res 711:163–174.

Zsombok A, Tóth Z, Gallyas F (2005) Basophilia, acidophilia and argyrophilia of ‘‘dark” (compacted) neurons during their formation, recovery or death in an otherwise undamaged environment. J Neurosci Methods 142:145–152.

(Received 11 May 2016, Accepted 4 November 2016)
(Available online 10 November 2016)